Ethnobotanical Leaflets 12: 758-62. 2008.

Medicinal Properties and Antimicrobial Activity of *Crotalaria madurensis* Var. *kurnoolica*

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Issued 01 October 2008

ABSTRACT

This paper deals with the antimicrobial and phytochemical studies of *Crotalaria madurensis* Wt. var. *kurnoolica* Ellis et Swaminathan. (Fabaceae), an endemic medicinal plant found in the forests of Nallamallias of Eastern Ghats of India. The ether and ethyl acetate extracts of the plant material exhibited a broad spectrum of antimicrobial activity on human pathogenic microorganisms of six bacterial and two fungal strains. The minimum inhibitory concentrations were provided. The results were supported by phytochemical analysis.

INTRODUCTION

Plant Material

Crotalaria madurensis Wt. var. *kurnoolica* Ellis et Swaminathan (Fabaceae, Vernacualar name – Adavijanumu) is an endemic species to Eastern Ghats, India, used for different ailments by adivasi tribes in the area. It grows occasionally along the hill slopes of Nallamalais, Kurnool district of Andhra Pradesh and it is endemic to Eastern Ghats (Venkata Raju & Pullaiah 1995). The ethno-medico-botanical studies of plant revealed that the plant is used by the Chenchu and Lambada tribe for the treatment of scabies. Fresh leaves crushed and paste applied externally and seeds were cooked and given in curry along with the food (Bhakshu, 2002). The biological and phytochemical studies of *Crotalaria madurensis* Wt. var. *kurnoolica* were hither to not reported and the results may provide wide applications in medicinal chemistry and pharmacological evaluation to develop novel antimicrobial drugs besides sustainable utilization and conservation of natural resources.

METHODS AND MATERIALS

The plant material was collected based on the information recorded from the local tribal practitioners by conducting repeated interviews. The leaves were collected from the Nallamalais hill ranges, dried in shade and were used for the present investigation. The voucher specimen (26967) was deposited at SKU (Sri Krishnadevaraya University, Anantapur) and identified by using authenticated floras (Venkata Raju & Pullaiah, 1995; Pullaiah *et al.*,

1997) and comparison with the authenticated specimens housed at SKU herbarium.

Qualitative Phytochemical studies

Hundred grams of shade dried leaves were pounded and extracted Successively using petroleum ether (60-80°C) Ethyl acetate (EtOAc) and Ethyl alcohol (EtOH) using a Soxhlet apparatus. The extracts yielded 300, 350 and 550 mg, respectively after evaporation in a roto-evaporator under the reduced pressure. Preliminary phytochemical studies were conducted on all extracts following the described procedures (Harborne, 1991; Chhabra 1984; Gibbs, 1974) and results were reported in table 1.

Table 1. Preliminary phytochemical studies of *C. madurensis* var. *kurnoolica* (leaves).

Phytochemical group	Pet. Ether Extract	Ethyl Acetate Extract	Ethyl alcohol Extract	Water extract
Alkaloids	-	+	+	-
Anthocyanins	+	+	-	-
Anthraquinones	-	-	++	+
Antracene glycosides	-	-	+	+
Catecholics compounds	-	-	+	++
Coumarins	-	Tr	+	-
Flavonoids	-	+	+	++
Lignans	-	+	+	-
Polyphenolics	-	-	+	+++
Reducing sugars	-	Tr	++	+++
Saponins	-	-	+	++
Steroids	++	+	-	-
Triterpenes	++	+	-	-
Volatile oils	+++	+	-	-

-, no reaction; tr, reaction in trace; +, indicates presence of compounds

ANTIMICROBIAL ASSAY

The agar disc diffusion method was used to determine the antimicrobial activity of the essential oil and crude extracts (Cruikshank, 1968). The discs (6 mm diameter) impregnated with known concentrations of the oil and extracts were placed on the surface of the Petri plates containing 20 ml of the respective media seeded with 0.1 ml of microbial suspensions (5×10^5 CFU/ml). Standard antibiotics viz., ampicillin, kanamycin, tetracycline and

vancomycin (30 µg/ disc) obtained from Hi-media, Mumbai, were used as positive controls. The plates were incubated for 24 hours at 35±2° C for bacteria and for 48 hours for yeasts at 30°C. The inhibition zones formed around the discs were measured and expressed in millimeters. Three independent trials were conducted for each concentration and the average values calculated and given in Table (2). The microbicidal activity was confirmed by transferring a sub-culture from the clear zone of inhibition to a fresh broth media and observed for the growth of microbes.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined, using a common broth micro dilution method in 96-well micro titer plates (Camporese et al., 2003; NCCLS, 1999). Two fold dilutions of each extract were carried out, starting from 5 to 0.15 mg/mL. 10 mL of the previously prepared different microbial suspensions (10^5 CFU/ mL) were added to each well. Plates were incubated for 18 h at37^0 C and then were examined with Elisa reader (TECAN, Sunrise, China) at 620 nm and the lowest concentration of each extract showing no growth was taken as its minimum inhibitory concentration (MIC). The solution DMSO (100 mL/mL) served as the negative control. All the samples were tested in triplicates to confirm the activity. All the samples were tested in triplicates to confirm the activity and the values were noted (Table 2).

Table 2. Antimicrobial activity of *C. madurensis* var. *kurnoolica* leaf extracts.

Test Microorganisms	Diameter of zone of inhibition (mm)			Minimum Inhibition Concentrations (MIC, µL / ml)	
	Pet. Ether extract (1mg/ml)	Et.Ac Extract (1mg/ml)	Standard Antibiotics (30 µg / disc)	Pet. Ether extract	Et.Ac Extract
Gram positive bacteria					
Bacillus subtilis MTCC121	-	14	22 [a]	-	500
Micrococcus luteus MTCC 1522	14	12	26 [b]	500	750
Staphylococcus aureus MTCC 737	14	14	24 [b]	500	500
Gram negative bacteria					
Escherichia coli MTCC1687	12	10	22 [b]	750	1000
Pseudomonas aeruginosa MTCC 1688	14	8	28 [b]	500	1000
Klebsiella pneumoniae MTCC 109	10	-	22 [b]	1000	1000
Proteus vulgaris					

MTCC 1771	-	-	24 [b]	-	-
Fungi					
Candida albicans MTCC183	10	10	24 [b]	1000	1000
Candida tropicalis MTCC187	12	10	22 [c]	1000	1000

EtAc: Ethyl Aceate; Standards: A: Ampicillin; b: Kanamycin; c: Tetracycline;

RESULTS AND DISCUSSION

The preliminary investigation of phytochemical studies (Table1) and antimicrobial activity (Table 2) were reported from the leaves of *Crotalaria madurensis* var *kurnoolica* for the first time. The petroleum ether and ethyl acetate extracts were proved as active against the tested microorganisms which exhibited a broad spectrum of antimicrobial activity. The ethyl alcohol and water extracts were failed to exhibit inhibitory property. *Proteus vulgaris is* resistant to the tested extracts at all concentrations. Based on the Minimum inhibitory concentrations (MIC) for active extracts represented (Table 2) the ether extract was effective against*M. luteus, S. aureus, P. aeruginosa, E. coli, K. pneumoniae* and *C. albicans*, While the ethyl acetate extract was active against *B. subtilis, M. luteus, S. aureus, E. coli, P. aeruginosa* and *C. albicans*. The MIC of petroleum ether extract of leaves was 500 µg against *M. luteus, S. aureus, P. aeruginosa* while 750 µg against *E. coli*, 750 µg on *K. pneumoniae, C. albicans* and *C. tropicalis*. The MIC of ethyl acetate extract was 500 µg against*S. aureus, B. subtilis, M. luteus*, and 750 µg against *C. albicans*, 1000 µg against *E. coli, P. aeruginosa* and *K. pneumoniae*.

CONCLUSIONS

Petroleum ether and ethyl acetate extracts of *C. madurensis* var. *kurnoolica* leaves were found to be active on the tested microorganisms whereas, the alcoholic extract did not show any inhibitory activity. Three Gram-positive (*B. subtilis*, *M. luteus* and *Staphylococcus aureus)*, two Gram-negative bacteria (*E. coli* and *P. aeruginosa)* and two fungal species (*C. albicans* and *C. tropicalis)* were observed to be sensitive to the tested extracts where as, *P. vulgaris* was found to be resistant. These results lend support the usage of *C. madurensis* var. *kurnoolica leaves* by the local tribal population in using for wounds and skin diseases against bacterial and fungal infections. Further studies are under way to isolate and characterize the major active principles of the oils and test the compounds on different microorganisms and against various infections, serve as a strong evidence for the plant as potent antimicrobial agent.

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